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Leptin receptor modulation of adiposity and fertility

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Abstract

The leptin receptor was discovered as a leptin binding protein, which is highly expressed in the choroid plexus. Mapping of the gene's chromosomal locations in rodents revealed that mutations in *Lepr* were the basis for obesity/diabetes mutations in rodents and humans. Genetic manipulations that target *Lepr* expression in specific neurons or hypothalamic areas have generated insights to the modes by which body composition and reproductive function are modulated by leptin receptor. These animal models have also been instrumental in identifying diabetes susceptibility genes. In this review, we discuss the evidence that supports the concept of networked functions of leptin receptor as it pertains to feeding, substrate utilization and reproduction.

Obesity of Leptin receptor deficiency caused by multiple mechanisms

The *diabetes* [1] and *fatty* [2] rodent models were established due to their extreme obesity, characterized by hyperphagia and elevated feed efficiency. Along with these traits, the mutant rodents showed a constellation of endocrinological disturbances: infertility due to hypothalamic hypogonadism, hyperinsulinemia associated with insulin resistance, hypercorticosteronemia, mild hypothyroidism and lower circulating growth hormone [3]. These models of obesity caused by mutations of the leptin receptor gene also exhibited a lowered core temperature [4], principally due to lowered heat production from atrophic brown adipose tissue [5]. Each of these traits affects body fat content and is therefore likely to contribute to the obese phenotype of leptin receptor deficiency. It is useful to compare the phenotypes of rodent and human LEPR-null mutants [6], specifically to address the applicability of findings in current rodent models to the human situation. The striking similarities and some notable differences in the phenotypic traits of leptin receptor deficits in rodents and humans make for an interesting comparison.

There are multiple underlying causes of obesity in leptin receptor mutants (Table 1). Most of these defects are likely due to defects in central nervous system regulation and to a major decrease in sympathetic nervous system outflow that promotes lipogenesis coupled with a failure to activate brown adipose tissue thermogenesis. We compare the phenotypes of rodent and human LEPR mutants in Table 1.

1. Hyperphagia is observed in all cases of leptin receptor deficiency and is the major cause of obesity in LEPR-null organisms. This excess caloric intake contributes to increased mass

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accumulation, and both hormonal and growth status dictate which tissues are affected (e.g. fat mass versus fat-free mass). The hyperphagia is readily observed during independent feeding episodes although increased intake during the suckling phase is not observed. While rodent pups, lean and genetically obese, are born nearly devoid of body fat, triglyceride deposition occurs rapidly in the first days of life. Leptin receptor deficiency causes an even more rapid triglyceride deposition in fat depots during this suckling phase, indicating that hyperphagia is not required for increased fat deposition [7]. If caloric intake is matched between lean and LEPR-null pups, the increased fat mass of the obese pups is counterbalanced by decreased lean mass accumulation.

2. Increased lipogenesis and decreased fat oxidation contribute to excess triglyceride accumulation in adipose tissue [8]. Interestingly, this preferential storage of fat is observed independent of caloric intake such that increased body fat content is relatively inert to caloric restriction or imposed exercise. This trait has been extensively demonstrated in LEPR-null rodents but not for LEPR-null humans. We predict this is likely to be the case for humans with LEPR-deficiency. In cases of growth hormone excess, increased caloric intake results in proportional somatic growth and reduced adiposity [9,10]; thus the case that positive energy balance invariably leads to fat accumulation does not appear to be universal.

3. *Decreased nonshivering thermogenesis* from brown adipose tissue, observed as decreased core temperature, significantly reduces the energy expenditure of LEPR-null rodents [11]. Comparisons of LEPR-null mice and wild type mice raised at thermoneutral and non-thermoneutral conditions indicate approximately a 25% difference in total energy dissipation due to the defect in nonshivering thermogenesis of LEPR-null mice [11]. LEPR-null rodents, with their increased adiposity potentially acting as insulation, are unable to adequately compensate for the defect in brown fat activation during cold stress. Despite the finding of functional brown adipose tissue in the neck region of humans [12], the major differences in the surface area to mass ratios between small rodents and humans make it unlikely that diminished nonshivering thermogenesis would make as large a contribution to the obesity of LEPR-null humans as it does in LEPR-null rodents.

4. *Diminished physical activity* has been documented in LEPR-null rodents [13]. Unfortunately, a quantification of spontaneous physical activity in terms of caloric equivalents has been elusive. There are severe technical constraints in measuring the energetic requirements for the movements of small rodents and the conversion of qualitative counts of locomotion to physical equivalents in actual thermodynamic units. Furthermore, limitations of experimental design are imposed by the physical environments of laboratory housed rodents. While the amount of spontaneous physical activity is rather small in terms of the overall energy budget, voluntary activity, which is unlikely to be influenced by leptin receptor activity or hypothalamic function, can greatly increase energy expenditure by several fold. Thus, the contribution of spontaneous physical activity to the obesity of LEPR-null rodents and humans remains an important but non-quantified term.

Leptin receptor signaling involves multiple signal transduction pathways

Leptin receptor isoforms, including several membrane bound receptors and a soluble receptor, are transcriptionally encoded or derived by proteolysis of membrane bound isoforms [14,15]. However, inactivation of the membrane bound isoform, LEPR-B, is sufficient to replicate the full phenotype of complete leptin or leptin receptor deficiency [16,17]. The LEPR-B isoform is the only leptin receptor isoform that contains both a janus kinase 2 (JAK2) binding domain and a signal transducer and activator of transcription 3 (STAT3) activation domain. The cytoplasmic region of LEPR-B (Figure 1) is formed from two exons, the coding exon 17 (encoding a 25 amino acid membrane proximal region containing the JAK2 binding domain) and alternative terminal coding exon 18b (encoding 273 amino acids including 3 tyrosine

phosphorylation sites: Y985, Y1077, and Y1138). The specific signal transducing molecules associated with these tyrosine phosphorylation sites have been identified and genetically manipulated in mice [18]. The Y1138 is necessary for STAT3 activation, and mice with an Y1138S mutant LEPR-B are obese and insulin resistant, yet fertility is retained [19]. However, these mice still remain unable to lactate, suggesting incomplete restoration of reproductive competence. The Y985 is required for recruitment of Src homology domain containing protein tyrosine phosphatase (SHP2) and suppressor of cytokine signaling 3 (SOCS3). Interestingly, female mice with a mutant Y985L LEPR are lean and resistant to diet induced obesity, indicating a major role for SOCS3 as an attenuator of leptin receptor signaling [20]. The activation of SHP2 and downstream ERK1/2 (extracellular signal-regulated kinase 1/2) is suggested to be important for leptin mediated proopiomelanocortin (POMC) neuron stimulation of sympathetic nervous system activity [21]. The Y1077 activates STAT5, although it is unclear whether leptin receptor signaling via Y1077 has a major impact on body weight or adiposity [22].

Leptin has demonstrable effects on neuronal excitability, typically acting within minutes, probably by acting on an unidentified mixed cation channel [23] that is found in a subset of neurons expressing POMC within the arcuate nucleus. We note that while acute effects of leptin on neuronal activity and feeding are affected by alterations of PI3K expression [24] presumably activated by JAK2, there is no long term impact on body weight or body composition due to loss of PI3K activity. This is in line with the observations that STAT3 activation is necessary for leptin's effects on metabolism and that neither LEPR isoforms that lack the STAT3 activation domain (LEPR-A in *db1J* mice) [16] nor a Y1138S mutant LEPR [19] prevent obesity associated with LEPR-B deficiency. All membrane bound LEPR isoforms contain part of the JAK2 binding site [14,15] and presumably are capable of partial phosphoinositide-3 kinase (PI3K) activation via JAK2. It is certainly possible that the short-term effects of leptin receptor activation, such as membrane depolarization, require protein synthesis and synaptic remodeling, actions that may be stimulated by transcriptional activation via STAT3, to attain long-term effects. This is reminiscent of the protein translational dependence of long term potentiation, suggesting that synaptic modeling is a requirement for the long-term effects of leptin receptor activation.

The potential for crosstalk between leptin and insulin signaling exists at multiple levels. Both hormones can activate PI3K as a direct consequence of binding to their respective receptors [25]. There is also potential overlap in transcriptional activation of common target genes by activation of transcriptional regulators: STAT3 for LEPR and forkhead box O1 (FOXO1) for insulin receptor (INSR). The promoter for two leptin target neuropeptide genes, *Agrp* and *Pomc*, contain bindings sites for both STAT3 and FOXO1 wherein squelching is possible due to the proximity of the binding sites to each other [26]. The prior discussion on PI3K and STAT3 highlights the complexity of leptin receptor signal transduction pathways within individual neurons. The presence of multiple, simultaneously active transduction pathways makes it imperative to ascertain the strengths and relevance of each pathway within a given neuron.

The development of obesity and leptin resistance

The initial discovery of leptin initiated a burst of activity examining the connection between leptin and adiposity in humans, leading to the observation that the amount of body fat is proportional to circulating leptin concentrations [27]. Thus, the concept of leptin resistance was put forth as an explanation for the ineffectiveness of leptin in reducing body fat in obese individuals without mutations in the leptin signaling axis [28]. Nevertheless, increasing leptin concentrations in the circulation or in the CNS of rodent models indicated that leptin, at high concentrations, is capable of decreasing food intake and body fat [29]. However, a late

complication of leptin overexpression is the precipitation of obesity upon the presentation of a calorically dense diet [30]. This observation is consistent with the concept that the leptin signaling pathway has components that have been shown to limit leptin signaling and, under conditions of prolonged exposure to high leptin concentrations, can lead to leptin insensitivity.

Molecular analyses of leptin receptor signal transduction pathways have led to insights into the regulation of leptin signaling. While LEPR is typically classified as a JAK-STAT activator, LEPR also has a tyrosine phosphorylation site (Y985) that can dock SHP2 and SOCS3 (Figure 1). A Y985L mutant LEPR [20] or deletion of SOCS3 in the brain [31] decreased both adiposity and resistance to body fat accumulation during high fat feeding. These observations indicate that a major contributor to diet induced obesity and leptin resistance comes from elevated leptin concentrations (caused by increased body fat) and attenuation of LEPR signal transduction by SOCS3. It is interesting to note that deletion of protein tyrosine phosphatase 1B (PTP1B), a phosphatase targeting JAK2, causes a lean phenotype under normal conditions but does not prevent obesity associated with high fat feeding [32]. Also, increasing LEPR numbers within neurons using LEPR transgenes does not alter body composition in mice [33], suggesting that synergistic interactions between dietary caloric density and leptin signal transduction intensity are pathogenetic mechanisms of obesity in humans. Further work will be necessary to ascertain whether these observations also apply to leptin receptor's modulation of type 2 diabetes mellitus and obesity-related infertility.

Identified neurons modulating food intake and substrate utilization

The observation that the leptin receptor induces the phosphorylation of STAT3 [34] has made it relatively easy to identify leptin activated neurons. One caveat is that some of these neurons might be activated indirectly as it is conceivable that STAT3 could become phosphorylated by activation from leptin sensitive neurons or that basal pSTAT3 is high in certain neurons. Nevertheless, this finding was of inordinate utility as highly sensitive anti-pSTAT3 antisera were available whereas there was a lack of high affinity LEPR-B specific antisera that could be used for visualization of LEPR in rodent brains. Treatment of mice with leptin, either systemically or intracerebrally, revealed large numbers of pSTAT3 bearing neurons in many hypothalamic and extra-hypothalamic sites [35]. We will restrict our discussion to identified neurons of known neurotransmitter phenotype that affect energy balance.

We know of two arcuate nucleus neuron types (Figure 2), proopiomelanocortin/cocaine-and amphetamine-regulated transcript (POMC/CART) neurons [36] and neuropeptide Y/agouti gene related peptide (NPY/AGRP) neurons that are regulated by leptin [13]. Increased POMC neuronal activity leads to decreased feeding and increased fat oxidation via the activation of melanocortin receptors 3 and 4 (MC3R and MC4R). Increased NPY/AGRP neuronal activity increases feeding and promotes fat deposition via activation of NPY receptors and AGRP mediated inverse agonism of MC3R and MC4R. Leptin signaling depolarizes and increases the firing rate of POMC/CART whereas it hyperpolarizes and decreases NPY/AGRP neuron activity. The ventral medial nucleus (VMN) contains leptin sensitive steroidogenic factor 1 (SF1)-expressing neurons [37] that synthesize brain derived neurotrophic factor (BDNF) (Figure 2). Loss of SF1 neuronal function leads to increased feeding and fat deposition. The lateral hypothalamic area neurons (LHA) express melanocyte concentrating hormone (MCH) (Figure 2) which are regulated by leptin and endocannabinoids [38]. Increased expression of MCH increases feeding whereas loss of MCH decreases feeding and protects from dietary fat accumulation [39]. Deletion of LEPR expression in each of the three cell types, POMC/CART [36], SF1 [37] and NPY/AGRP [13], provides evidence that each neuronal type contributes to a fraction of the obesity of complete LEPR signaling deficiency. Interestingly, each of these neuronal types sends and/or receives projections from at least one other neuronal type, suggesting a highly distributed network of neurons. This form of distributed network contrasts

strongly with the neuroendocrine system regulating reproductive function which pivots around one critical cell type, the gonadotropin-releasing hormone (GnRH) neuron (discussed below).

Insulin resistance and diabetes susceptibility – Potential species differences

It has been difficult to define the neural pathways and contributing neurons that mediate leptin's effects on insulin sensitivity. Nevertheless, it is clear that descending projections [40], most likely from the hypothalamus, have a major impact on hepatic glucose production. Some of the specific neurons that are involved reside within the arcuate nucleus, with contributions from POMC [41,42] and AGRP/NPY [43] neurons. Contributions from other types of neurons await further definition. As type 2 diabetes mellitus is currently considered to be the result of insulin resistance and a defect in pancreatic β -cell function affecting insulin release, it is reasonable to propose that the descending projections from the hypothalamus affects both insulin release and insulin sensitivity, both of which are severely dysfunctional in LEPR-null rodent models. Additional genetic factors would provide the deciding factor in the ultimate susceptibility of a specific inbred strain to develop hyperglycemia.

The original mutant *Lepr* locus was termed *diabetes (db1J)* due to the extreme hyperglycemia of the original mutant mice, *db1J* homozygotes on the C57BLKS/J strain [1]. While the *db1J* homozygous mice were obese, their hyperglycemia distinguished them from mice carrying another mutation called *obese*, subsequently found to be the locus that encoded the leptin gene. Further genetic analysis revealed that the diabetes susceptibility varied between various genetic strains [44].

A similar strain dependence on diabetes susceptibility has been reported for mutations of the rat *LEPR* locus. The rat *LEPR* mutations, *fatty* [2] and *fatty-Koletsky* [45], originated on two different outbred strains. The *fatty* homozygotes of the original 13M strain are typically euglycemic but insulin resistant. The *fatty-Koletsky* homozygous rats of an outbred strain background are uniformly hyperglycemic and show typical complications of long-term hyperglycemia. Subsequent congenic strain production from the two rat *LEPR* mutant alleles have shown that insulin resistance is common to all *LEPR*-null rats [46,47], whereas hyperglycemia development depends on genetic background, similar to the pattern in mice.

Two genes have been identified that are responsible for some of these strain-specific susceptibilities to diabetes: *Sorsc1* [48] and *Lisch-like* [49]. Both of these genes were identified by laborious and near decades-long positional cloning strategies. Based on the observations that various inbred mouse strains show differential susceptibilities to develop hyperglycemia in the context of leptin or leptin receptor deficiency, multiple subcongenic lines were developed to delineate the critical intervals that contained the differential susceptibility genes to manageable sub-centimorgan regions. The genes were identified after an exhaustive screen of all genes within the critical intervals. Not surprisingly, both genes are normally expressed in islets and/or β -cells, and for both cases, the susceptible alleles had weak expression of the normal transcript and acted effectively as a null or hypomorphic allele.

The most extensive characterization of *LEPR*-null humans has documented insulin resistance and variable development of type 2 diabetes mellitus from 7 families [6]. However, the severity of hyperglycemia is remarkably mild with only two *LEPR*-null individuals developing blood glucose values above 115 mg/dl. This was an age-dependent phenomenon: two individuals aged 41 and 55 years developed type 2 diabetes mellitus, whereas the other 8 *LEPR*-null cases that ranged between 4 and 31 years of age were euglycemic.

This striking difference between the diabetes phenotypes of *LEPR*-null rodents and humans (Table 1) can be ascribed to either a species difference in the contribution of the liver to glucose metabolism or in differences in the neuronal network controlling glucose homeostasis. While

we do not have any evidence with regard to species differences in leptin-responsive neuronal networks controlling glucose metabolism, there is a clear difference in basal hepatic glucose output between mice and humans. Mice [50] and rats [51] have at least a 4-fold increase (~10 mg/kg/min) in basal hepatic glucose output over humans (~2 mg/kg/min) [52], placing a premium on tight regulation of glucose output in rodents. Therefore, the impact of alterations in any facet of glucose metabolism is magnified in rodents when compared to humans. Indeed, a review of the various rodent strains wherein leptin receptor mutations have been characterized suggests that hyperglycemia is the typical outcome for rodents with defective leptin receptor signaling.

Hypothalamic hypogonadism and infertility of LEPR-deficiency

Rodents and humans with leptin receptor deficiency have hypothalamic hypogonadism, resulting in delayed pubertal development and infertility. Female rodents and humans [6] with deficient leptin receptor signaling have atrophic uterine and ovarian size, abnormal estrous cyclicity and impaired mammary gland morphology and function [53]. Male mice with deficient leptin signaling have atrophic testicles and impaired spermatogenesis and behavioral responses to normal receptive females [54]. While leptin supplementation was initially suggested to be an initiator of puberty, subsequent tests of this hypothesis indicated that leptin acts mainly as a permissive factor [55].

Although leptin signaling contributes to regulation of the hypothalamic-pituitary-gonadal axis, leptin receptors (Figure 3) are not expressed on GnRH neurons [56], which are absolutely essential for normal reproductive function. Therefore, leptin must act indirectly to regulate GnRH neuron activity via its downstream targets, including NPY, AGRP, POMC, kisspeptin, and nitric oxide synthase (NOS), all of which modulate GnRH neuron activity and/or gonadotropin levels [57]. As previously mentioned, it appears that the transcriptional effects of leptin receptor signaling are not necessary for leptin's modulatory effects on GnRH neurons since ablation of STAT3 activation by LEPR does not impose infertility in Y1138S LEPR-B mutant females [19].

Within the arcuate nucleus, there are three types of neurons that have demonstrable effects on GnRH release: NPY/AGRP, POMC/CART and KISS1 neurons (Figure 3). The NPY/AGRP neuron is inhibited by leptin receptor signaling and has been suggested to be an inhibitor of GnRH release. Both NPY and AGRP [58] are overexpressed in leptin signaling defective states, increasing inhibitory inputs to GnRH neurons. Additionally, the loss of NPY in *ob/ob* female mice improves the fertility and lactational competence in these mice. Ablation of the NPY receptor, Y4R, in *ob/ob* females also improves the infertility but, unlike NPY loss, Y4R knockout does not improve mammary gland development and function. Thus, low leptin states, such as undernutrition or enhanced NPY/AGRP neuronal activity, would mimic the LEPR-null state and adversely affect the reproductive system with the potential of altering pubertal progression.

Melanocortins, released by LEPR-bearing POMC neurons of the arcuate nucleus, are also involved in regulating GnRH neurons. Melanocortin agonist treatment can induce GnRH and luteinizing hormone (LH) release, as well as accelerate GnRH pulse activity whereas antagonist treatment can block these effects [59,60]. Although our original report about mice with LEPR deletion in POMC and AGRP/NPY neurons indicated normal fertility at relatively young ages, we have noted diminished fertility as the mice age. Further studies are warranted to assess the impact of melanocortinergic transmission on GnRH neurons and reproduction.

Kisspeptin neurons (Figure 3) have a major impact on GnRH neurons as loss of KISS1 peptide or its receptor, GPR54, results in hypothalamic hypogonadism and infertility [61]. KISS1 neurons of the arcuate nucleus are considered to be stimulatory to GnRH release. Kisspeptin

gene expression within the ARC is also stimulated by leptin [62]. Within the ARC, KISS1 neurons also co-express dynorphin A and neurokinin B. While loss of Neurokinin B, also known as TAC3 (tachykinin 3), and its receptor also causes hypothalamic hypogonadism in humans [63], the role of dynorphin A, an endogenous opioid, in reproduction is unclear. KISS1 neurons are also found in other hypothalamic sites responsible for reproduction, the dorsomedial nucleus (DMN) and anteroventral periventricular nucleus (AVPV), although these neurons have not been demonstrated to be regulated by leptin.

The ventral premammillary nucleus (PMv) (Figure 3) has emerged as an important site for leptin mediated effects on reproductive function. In response to both leptin as well as olfactory cues, sex steroid sensitive PMv neurons that project onto GnRH neurons stimulate gonadotropin release and modulate sexual behavior [64]. Bilateral lesioning of the PMv in rodents causes diminished fertility and decreased GnRH, gonadotropin and sex steroid levels as well as preventing leptin mediated LH secretion in fasted mice [65]. As these pheromone sensitive neurons of the PMv include those expressing target CART and nNOS [66], the reproductive defects in nNOS knockout mice [67] should be considered evidence for the role of these neurons in mediating leptin's impact on GnRH neuronal function.

Summary

We have provided a view of a leptin-regulated network of neurons that provide a coordinated response to control feeding behavior, substrate oxidation and reproductive function. More neuron types remain to be identified to complete the cast of players in this network. An even greater task is to define the interconnectivity of these separate neuron types and the coordination of their outputs. A similar puzzle of defining the interactions between various hormonal and nutrient signaling pathways within the components of the network needs to be solved to understand the control of metabolism and reproduction. Within the genome, diabetes modifier genes remain to be identified while pathways that control β -cell proliferation/survival and inflammatory responses are major factors in causing hyperglycemia.

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References

1. Hummel KP, et al. Diabetes, a new mutation in the mouse. *Science* 1966;153:1127–1128. [PubMed: 5918576]
2. Zucker L, Zucker T. Fatty, a new mutation in the rat. *J Heredity* 1961;52:275–278.
3. Leibel, R., et al. Obesity.. In: Scriver, C., et al., editors. *The metabolic and molecular bases of inherited disease*. Eighth edition. McGraw-Hill; 2001. p. 3965-4028.
4. Trayhurn P. Thermoregulation in the diabetic-obese (db/db) mouse. The role of non-shivering thermogenesis in energy balance. *Pflugers Arch* 1979;380:227–232. [PubMed: 573463]
5. Cusin I, et al. Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. *Diabetes* 1998;47:1014–1019. [PubMed: 9648822]
6. Farooqi IS, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med* 2007;356:237–247. [PubMed: 17229951]
7. Buchberger P, Schmidt I. Is the onset of obesity in suckling fa/fa rats linked to a potentially larger milk intake? *Am J Physiol* 1996;271:R472–476. [PubMed: 8770150]
8. Bazin R, Lavau M. Development of hepatic and adipose tissue lipogenic enzymes and insulinemia during suckling and weaning on to a high-fat diet in Zucker rats. *J Lipid Res* 1982;23:839–849. [PubMed: 6752318]

9. Katznelson L. Alterations in body composition in acromegaly. *Pituitary* 2009;12:136–142. [PubMed: 18369725]
10. Palmiter RD, et al. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* 1982;300:611–615. [PubMed: 6958982]
11. Trayhurn P, Fuller L. The development of obesity in genetically diabetic-obese (db/db) mice pair-fed with lean siblings. The importance of thermoregulatory thermogenesis. *Diabetologia* 1980;19:148–153. [PubMed: 7418967]
12. Cypess AM, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360:1509–1517. [PubMed: 19357406]
13. van de Wall E, et al. Collective and individual functions of leptin receptor modulated neurons controlling metabolism and ingestion. *Endocrinology* 2008;149:1773–1785. [PubMed: 18162515]
14. Chua SC Jr. et al. Fine structure of the murine leptin receptor gene: splice site suppression is required to form two alternatively spliced transcripts. *Genomics* 1997;45:264–270. [PubMed: 9344648]
15. Chung WK, et al. Exonic and intronic sequence variation in the human leptin receptor gene (LEPR). *Diabetes* 1997;46:1509–1511. [PubMed: 9287054]
16. Lee GH, et al. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 1996;379:632–635. [PubMed: 8628397]
17. Tartaglia LA, et al. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995;83:1263–1271. [PubMed: 8548812]
18. Myers MG, et al. Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol* 2008;70:537–556. [PubMed: 17937601]
19. Bates SH, et al. STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 2003;421:856–859. [PubMed: 12594516]
20. Bjornholm M, et al. Mice lacking inhibitory leptin receptor signals are lean with normal endocrine function. *J Clin Invest* 2007;117:1354–1360. [PubMed: 17415414]
21. Rahmouni K, et al. Hypothalamic ERK mediates the anorectic and thermogenic sympathetic effects of leptin. *Diabetes* 2009;58:536–542. [PubMed: 19066310]
22. Villanueva EC, Myers MG Jr. Leptin receptor signaling and the regulation of mammalian physiology. *Int J Obes (Lond)* 2008;32(Suppl 7):S8–12. [PubMed: 19136996]
23. Cowley MA, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 2001;411:480–484. [PubMed: 11373681]
24. Hill JW, et al. Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. *J Clin Invest* 2008;118:1796–1805. [PubMed: 18382766]
25. Xu AW, et al. PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 2005;115:951–958. [PubMed: 15761497]
26. Kitamura T, et al. Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake. *Nat Med* 2006;12:534–540. [PubMed: 16604086]
27. Rosenbaum M, Leibel RL. The role of leptin in human physiology. *N Engl J Med* 1999;341:913–915. [PubMed: 10486426]
28. Caro JF, et al. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996;348:159–161. [PubMed: 8684156]
29. Yura S, et al. Accelerated puberty and late-onset hypothalamic hypogonadism in female transgenic skinny mice overexpressing leptin. *J Clin Invest* 2000;105:749–755. [PubMed: 10727443]
30. Ogus S, et al. Hyperleptinemia precipitates diet-induced obesity in transgenic mice overexpressing leptin. *Endocrinology* 2003;144:2865–2869. [PubMed: 12810541]
31. Mori H, et al. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 2004;10:739–743. [PubMed: 15208705]
32. Bence KK, et al. Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med* 2006;12:917–924. [PubMed: 16845389]
33. de Luca C, et al. Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. *J Clin Invest* 2005;115:3484–3493. [PubMed: 16284652]
34. Vaisse C, et al. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 1996;14:95–97. [PubMed: 8782827]

35. Elias CF, et al. Chemical characterization of leptin-activated neurons in the rat brain. *J Comp Neurol* 2000;423:261–281. [PubMed: 10867658]
36. Balthasar N, et al. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* 2004;42:983–991. [PubMed: 15207242]
37. Dhillon H, et al. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 2006;49:191–203. [PubMed: 16423694]
38. Jo YH, et al. Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. *Neuron* 2005;48:1055–1066. [PubMed: 16364907]
39. Pissios P. Animals models of MCH function and what they can tell us about its role in energy balance. *Peptides*. 2009 In press.
40. Pocai A, et al. A brain-liver circuit regulates glucose homeostasis. *Cell Metab* 2005;1:53–61. [PubMed: 16054044]
41. Mizuno TM, et al. Transgenic neuronal expression of proopiomelanocortin attenuates hyperphagic response to fasting and reverses metabolic impairments in leptin-deficient obese mice. *Diabetes* 2003;52:2675–2683. [PubMed: 14578285]
42. Savontaus E, et al. Metabolic effects of transgenic melanocyte-stimulating hormone overexpression in lean and obese mice. *Endocrinology* 2004;145:3881–3891. [PubMed: 15117873]
43. Konner AC, et al. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. *Cell Metab* 2007;5:438–449. [PubMed: 17550779]
44. Leiter EH, et al. The influence of genetic background on the expression of mutations at the diabetes locus in the mouse. III. Effect of H-2 haplotype and sex. *Diabetes* 1981;30:1029–1034. [PubMed: 7030828]
45. Koletsky S. Obese spontaneously hypertensive rats--a model for study of atherosclerosis. *Exp Mol Pathol* 1973;19:53–60. [PubMed: 4721724]
46. Pederson RA, et al. Comparison of the enteroinsular axis in two strains of obese rat, the fatty Zucker and the JCR:LA-corpulent. *Int J Obes* 1991;15:461–470. [PubMed: 1894423]
47. Timmers KI, et al. Genetically obese rats with (SHR/N-cp) and without diabetes (LA/N-cp) share abnormal islet responses to glucose. *Metabolism* 1992;41:1125–1133. [PubMed: 1406299]
48. Clee SM, et al. Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. *Nat Genet* 2006;38:688–693. [PubMed: 16682971]
49. Dokmanovic-Chouinard M, et al. Positional cloning of “Lisch-Like”, a candidate modifier of susceptibility to type 2 diabetes in mice. *PLoS Genet* 2008;4:e1000137. [PubMed: 18654634]
50. Okamoto H, et al. Restoration of liver insulin signaling in Insr knockout mice fails to normalize hepatic insulin action. *J Clin Invest* 2005;115:1314–1322. [PubMed: 15864351]
51. Rossetti L, et al. Mechanism by which hyperglycemia inhibits hepatic glucose production in conscious rats. Implications for the pathophysiology of fasting hyperglycemia in diabetes. *J Clin Invest* 1993;92:1126–1134. [PubMed: 8397219]
52. Hundal RS, et al. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* 2002;109:1321–1326. [PubMed: 12021247]
53. Marin Bivens CL, Olster DH. Abnormal estrous cyclicity and behavioral hyporesponsiveness to ovarian hormones in genetically obese Zucker female rats. *Endocrinology* 1997;138:143–148. [PubMed: 8977397]
54. Batt RA, et al. Investigation into the hypogonadism of the obese mouse (genotype ob/ob). *J Reprod Fertil* 1982;64:363–371. [PubMed: 6175745]
55. Bronson FH. Puberty in female mice is not associated with increases in either body fat or leptin. *Endocrinology* 2001;142:4758–4761. [PubMed: 11606441]
56. Quenell JH, et al. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology* 2009;150:2805–2812. [PubMed: 19179437]
57. Ojeda SR, et al. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology* 2006;147:1166–1174. [PubMed: 16373420]
58. Korner J, et al. Leptin regulation of AgRP and Npy mRNA in the rat hypothalamus. *J Neuroendocrinol* 2001;13:959–966. [PubMed: 11737554]

59. Matsuyama S, et al. Activation of melanocortin receptors accelerates the gonadotropin-releasing hormone pulse generator activity in goats. *Neurosci Lett* 2005;383:289–294. [PubMed: 15955423]
60. Stanley SA, et al. gamma-MSH increases intracellular cAMP accumulation and GnRH release in vitro and LH release in vivo. *FEBS Lett* 2003;543:66–70. [PubMed: 12753907]
61. Dungan HM, et al. The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonadotropin-releasing hormone/luteinizing hormone. *J Neurosci* 2007;27:12088–12095. [PubMed: 17978050]
62. Smith JT, et al. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *J Neuroendocrinol* 2006;18:298–303. [PubMed: 16503925]
63. Topaloglu AK, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet* 2009;41:354–358. [PubMed: 19079066]
64. Leshan RL, et al. Direct innervation of GnRH neurons by metabolic- and sexual odorant-sensing leptin receptor neurons in the hypothalamic ventral premammillary nucleus. *J Neurosci* 2009;29:3138–3147. [PubMed: 19279251]
65. Donato J Jr. et al. The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. *J Neurosci* 2009;29:5240–5250. [PubMed: 19386920]
66. Sliwowska JH, et al. The premammillary hypothalamic area of the ewe: anatomical characterization of a melatonin target area mediating seasonal reproduction. *Biol Reprod* 2004;70:1768–1775. [PubMed: 14973262]
67. Gyurko R, et al. Deletion of exon 6 of the neuronal nitric oxide synthase gene in mice results in hypogonadism and infertility. *Endocrinology* 2002;143:2767–2774. [PubMed: 12072412]

Figure 1.

Signal transduction mechanisms of leptin receptor (LEPR-B). Leptin binding to a pre-dimerized leptin receptor molecule activates JAK2, Janus Kinase 2, leading to phosphorylation of three tyrosine residues on the cytoplasmic region of LEPR. JAK2 activation also leads to PI3K (Phosphatidylinositol 3-Kinase) activation and rapid, transient effects on neurons. Y985 is a binding site for SHP2 (Src homology domain protein tyrosine phosphatase 2) and SOCS3 (Suppressor of cytokine signaling 3). The binding of SOCS3 to Y985 is important to attenuate LEPR signaling and the development of leptin resistance. Y1138 recruits STAT3 for phosphorylation which increases nuclear localization of pSTAT3. STAT3 increases transcription of its target genes, among which are SOCS3, POMC (proopiomelanocortin) and AGRP (agouti gene related peptide). Other target genes apparently are necessary for the long term effects of leptin on controlling food intake and substrate oxidation.

BODY MASS AND BODY COMPOSITION

Figure 2.

Neural network of LEPR neurons regulating body mass and body composition. Three areas of the hypothalamus are denoted: ARC (arcuate nucleus), VMN (ventromedial nucleus) and the lateral hypothalamic area (LHA). Chemically identified neurons (NPY/AGRP, POMC/CART, SF1 (steroidogenic factor 1) and MCH (melanocyte concentrating hormone)) are denoted by their known neurotransmitters and receptors. Arrows are used to show synaptic connections between neuronal types with yellow arrows indicating an inhibitory/hyperpolarizing projection and a black arrow denoting a stimulatory/depolarizing projection. LEPR bearing neurons within the ARC send projections to MC4R expressing neurons within the paraventricular nucleus (PVN) that regulate feeding, while the significance of other melanocortinergeric projections from these ARC neurons remains to be determined.

REPRODUCTION

Figure 3.

Neural network of LEPR neurons regulating reproduction. Two areas of the hypothalamus containing LEPR neurons that are chemically identified are shown: ARC and PMv (ventral pre-mammillary nucleus). The identified neurons (NPY/AGRP, POMC/CART, KISS1 (kisspeptin) and CART/nNOS (cocaine amphetamine regulated transcript/neuronal nitric oxide synthase)) are marked with their known neurotransmitters and receptors. Yellow arrows indicate inhibitory/hyperpolarizing projections, and black arrows denote stimulatory/depolarizing projections. All of the specified neurons send projections to gonadotrophin releasing hormone (GnRH) neurons within the pre-optic area (POA), the critical regulator of the pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH).

Table 1

Cross species comparison of phenotypes between rodents and humans with leptin receptor mutations

Obesity	Rodents	Humans
Hyperphagia	Yes	Yes
Increased Lipogenesis	Yes	Not known
Increased Feed Efficiency	Yes	Not known
Low Core Temperature	Yes	Not known
Lower physical activity	Yes	Not known
Diabetes		
Insulin Resistance	Severe	Mild
Pancreatic β -cell loss	Strain dependent	Not known
Hyperglycemia	Yes	Variable
Infertility		
Delayed/abnormal Puberty	Yes	Yes
Low Gonadotropins	Yes	Yes